

Morphology and Ultrastructure of the Pharynx in Solenofilomorphidae (Acoela)

Christiane Todt* and Seth Tyler

Department of Biological Sciences, University of Maine, Orono, Maine 04469

ABSTRACT The homology of pharynges within the mostly pharynx-less Acoela has been a matter of discussion for decades. Here, we analyze the pharynges of three members of the Solenofilomorphidae, *Myopea* sp. and two species of the genus *Solenofilomorpha*, by means of light and transmission electron microscopy. Special focus is placed on the ultrastructure of the pharyngeal musculature, epidermis surrounding the mouth, pharyngeal epithelium, and junction with the digestive parenchyma. The main goal of this study was to evaluate the usefulness of certain characters for broader comparisons within the Acoela. Among the three species, characters relating to position of the mouth, presence and elaboration of sphincter muscles, presence of pharyngeal glands, and ultrastructure of epitheliosomes proved to be variously species- and genus-specific. The arrangement of pharyngeal muscles and their connection with body wall musculature, ultrastructure of receptor cells, and morphology of a non-ciliated glandular region in the posterior pharynx, in contrast, appear to be characteristic of the family Solenofilomorphidae and thus of predominant interest for comparisons with other acoel families. *J. Morphol.* 000: 000–000, 2006. © 2006 Wiley-Liss, Inc.

KEY WORDS: Acoelomorpha; foregut; fine structure; cilia; musculature; digestive parenchyma

The Solenofilomorphidae stands out within the Acoela in that its members bear a distinct and mostly muscular pharynx (Fig. 1A,B), a character present in only a few other acoel taxa. The general morphology and histology of pharynges of representatives of the five solenofilomorphid genera have been described in an extensive monograph of the Solenofilomorphidae (Crezée, 1975; see also Dörjes, 1971). The solenofilomorphid pharynx is generally considered to be a pharynx simplex (sensu Hyman, 1951; Doe, 1981; Ax, 1996), derived from a simple terminal or ventral invagination of the body wall. For this type of pharynx, Riedl (1954) further distinguished the pseudopharynx, composed of body wall elements only, from the more complicated archipharynx, with “autochthonous” (parenchymal?) elements such as additional circular muscle layers or sphincters. Most authors (Ax, 1961; Steinböck, 1966; Crezée, 1975) have not followed this terminology on the grounds that such a distinction is hard to make without actual embryological data.

The solenofilomorphid pharynx is lined by a ciliated insunk epithelium having cilia more closely spaced than those of the epidermis (Doe, 1981). All solenofilomorphid pharyngeal epithelium is described as ciliated in contrast to the condition in other acoels, which have a terminal, nonciliated region (specifically, *Hofstenia* [Bock, 1923, for *H. atroviridis*], *Nadina* [Riedl, 1954, for *N. pulchella*], *Oligochoerus* [Ax and Dörjes, 1966, for *O. limnophilus*], and *Diopisthoporus* [Dörjes, 1968 for *D. brachypharyngeus*]). All pharyngeal cells in solenofilomorphids bear eosinophilic vesicles and thus obviously have secretory functions, but additional long-necked unicellular pharynx glands are restricted to representatives of *Myopea* and *Oligofilomorpha*.

The pharynx musculature is described as consisting of an inner (adluminal) layer of circular muscles and an outer (abluminal) layer of longitudinal muscles, with the exception of *Endocincta*, which exhibits no circular muscles but instead possesses adluminal spiral muscles (Crezée, 1975). Sphincter muscles may be present at the mouth opening and at the posterior opening into the digestive parenchyma, and the degree of muscularization here differs considerably among genera. Despite these variations, one common feature is that the longitudinal muscle fibers of the body wall pass by the mouth opening and do not take part in pharynx formation. The circular body wall muscles near the mouth in *Myopea*, *Solenofilomorpha*, and *Fusantrum*, however, extend into the pharynx to form its longitudinal musculature. The adluminal circular muscle layer of the pharynx is thus interpreted as an autochthonous element developed independently from the body wall musculature (Crezée, 1975). This is in

Contract grant sponsor: National Science Foundation; Contract grant number: 0118804; Contract grant sponsor: Austrian Science Fund; Contract grant number: J2423-BO3 (Erwin Schrödinger Fellowship to C.T.).

*Correspondence to: Christiane Todt, Department of Biological Sciences, The University of Maine, 5751 Murray Hall, Orono, ME 04469. E-mail: christiane@todt.or.at

Published online in
Wiley InterScience (www.interscience.wiley.com)
DOI: 10.1002/jmor.10440

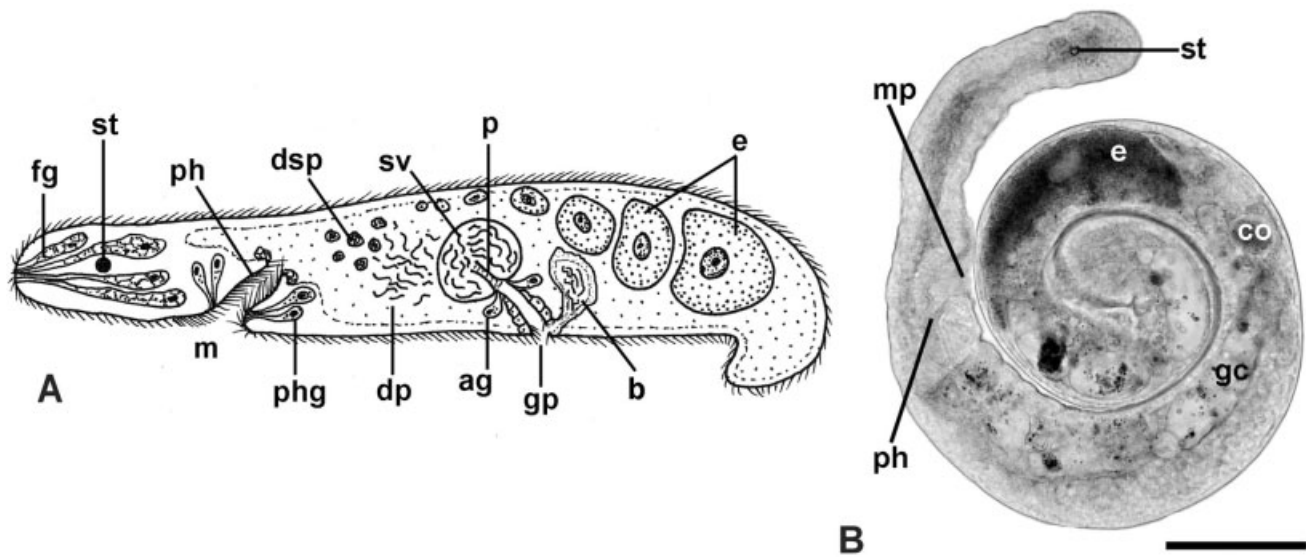


Fig. 1. Body plan of Solenofilomorphidae. **A:** *Myopea*, schematic drawing in a lateral view. The anterior body, to the left, shows frontal glands (fg), statocyst (st), and the mouth (m) and pharynx (ph) with pharyngeal glands (phg). The posterior body, to the right, bears eggs (e), developing sperm (ds), copulatory organ composed of seminal vesicle (sv), penis (p), accessory glands (ag) of the antrum, the female bursa (b), and common gonopore (gp). **B:** *Solenofilomorpha* sp. 2. Micrograph of living specimen, posterior body tightly curled. Scale bar = 300 μm . co, copulatory organ; e, egg; gc, gut contents; mp, mouth; ph, pharynx; st, statocyst.

strict contrast to the situation in other acoel taxa, like Proporidae, Hofsteniidae, and Diopisthoporidae, where the longitudinal body wall muscles extend into the pharynx to form its longitudinal musculature and the layer of circular body wall muscles is continuous with the circular pharynx muscles. The only other known example within the Acoela with circular body wall muscles turning into the pharynx is *Nadina pulchella*, but there the longitudinal body wall fibers are also continuous with longitudinal pharyngeal fibers (Riedl, 1954).

As demonstrated for the pharynx simplex of a wide range of turbellarian species of the Catenulida, Haplopharyngida, and Macrostomida, and two acoel species (Doe, 1981), there are certain pharynx characters, including several ultrastructural features, that are of value for phylogenetic considerations. These include the pharynx position, structure of the pharyngeal nervous system, receptor-cell cytology, pharynx region specialization, pharynx musculature, cytological features of epithelial cells (e.g., nuclei, microvilli, mitochondria, cilia), and ciliary rootlet orientation. Many ultrastructural features are evolutionarily conserved and are not as strongly influenced by feeding mechanisms as are most morphological characters detected by light microscopy. Currently, however, there is little available information on the ultrastructure of acoel pharynges. Crezée (1975) shows transmission electron microscopy (TEM) images of the pharynx epithelium of *Endocincta punctata*, a solenofilomorphid with a comparatively small and weakly muscled pharynx. Doe (1981) studied the epidermis around the mouth and the epithelia of the mouth and pharynx in an unde-

termined species of *Diopisthoporus* and in *Solenofilomorpha funilis*.

Here, we investigate the pharynges of three solenofilomorphid species, *Myopea* sp. and two species of *Solenofilomorpha*, using light microscopy and TEM. For each species the following features are considered: location and general morphology of the mouth and pharynx; ultrastructure of the pharyngeal musculature; ultrastructure of the ventral epidermis surrounding the mouth in comparison to the oral cavity and pharyngeal epithelia; ultrastructure of receptor cells in the mouth and pharynx; ultrastructure of pharyngeal gland cells (if present); and ultrastructural features of the junction of the pharyngeal epithelium with the digestive parenchyma (syncytium) and the digestive cells near the pharynx.

Our results, together with the available data on *Endocincta punctata* and *Solenofilomorpha funilis*, provide a good basis for comparison of ultrastructural features within the Solenofilomorphidae. This facilitates further evaluation of pharyngeal characters with respect to their importance for phylogenetic considerations and thus is crucial for further studies focused on the homology of acoel pharynges.

MATERIALS AND METHODS

Specimens of *Myopea* sp. were collected in shallow subtidal fine sands at Crow Neck (Maine). *Solenofilomorpha* sp. 1 and *Solenofilomorpha* sp. 2 came from similar sediments at Bakeman's beach (Maine). They were extracted from the substrate after anesthetization in an aqueous solution of magnesium sulfate made isotonic to seawater (~7.14%) following the procedures described by Sterrer (1971).

For TEM, specimens were anesthetized in magnesium chloride solution, prefixed 2 h in cold 5% glutaraldehyde in a 0.2 M sodium-cacodylate buffer, pH 7.3, with 12% sucrose and 0.78% NaCl, and postfixed for 1 h in a cold solution of 1% osmium tetroxide in distilled water. They were washed in distilled water, dehydrated in an acetone series, and embedded in Epon-Araldite epoxy resin. Semithin (2 μm) section series and ultrathin (75 nm) sections were produced. Semithin sections were stained with Toluidine blue; ultrathin sections were contrasted with uranyl acetate and lead citrate and examined with a Philips CM10 transmission electron microscope.

TEM micrographs were digitized, edited for contrast with Adobe Photoshop 7.0 (San Jose, CA), and image plates were produced with Adobe Illustrator 10.

A relative scale was used for the position of the mouth pore following Rieger and Sterrer (1968). Thereby, the body length is taken as 100 units (U), U0 being at the anterior body tip of the animal and U100 at the posterior tip. In an animal with the mouth exactly in midbody, for example, the mouth is at U50 (at 50% of the total length).

RESULTS

General Morphology of the Mouth and Pharynx

The tubular pharynx opens by way of a ventral mouth pore that is close to the anterior body tip in *Myopea* sp., but in a somewhat more posterior position in both the *Solenofilomorpha* species (Fig. 2A,C,E). Mouth and pharynx are surrounded by a dense network of adluminal (central) longitudinal and abluminal (peripheral) circular muscle fibers. The density of fibers within this network is considerably higher than in the body wall musculature, and individual muscle fibers are thin, with the exception of a few strong sphincter fibers at the mouth opening or at the junction with the digestive parenchyma. Longitudinal body wall muscles pass by the mouth pore but some specialized circular body wall muscles run into the pharynx and form its longitudinal musculature. Both mouth and pharynx are highly expandable and this high plasticity in life has to be considered when giving measurements. In fixed specimens, however, musculature tends to be contracted, and thus sizes given here most likely reflect minimum rather than maximum values.

***Myopea* sp.** The mouth is a longitudinally elongate slit with the actual mouth pore at U10–12. Pharyngeal and mouth epithelia are similar but are clearly distinct from the bordering epidermis (for details, see below). The specialized epithelium at the mouth narrows to an elongate furrow that extends to the anterior body tip. Thus, a frontal mouth area is present, which may be about as long as the pharynx itself when the mouth and pharyngeal musculature are relaxed (Fig. 2A). In an adult specimen with a body length of 1.17 mm after fixation, for example, the frontal mouth area was 155 μm long, the anterior pharynx wall 145 μm long, and the posterior pharynx wall 120 μm long. There are a few strong sphincter fibers surrounding the mouth which may close it and retract part of the frontal mouth area to form a dorsal vault of the mouth/

pharyngeal cavity. The large cell bodies of long-necked serous gland cells are situated lateral to the posterior pharyngeal wall (Fig. 2B). Their slender necks turn anteriorly, then they pass through the pharyngeal musculature, and their serous secretions are discharged into the anterior part of the pharynx (for details, see below). A few thick sphincter fibers are situated at the posterior end of the pharynx, where the pharyngeal epithelium borders the digestive parenchyma.

***Solenofilomorpha*.** The mouth pore of *Solenofilomorpha* sp. 1 is located at U14–17. In specimens of 1.25 mm body length, the anterior pharynx wall is about 250 μm long and the posterior wall 200 μm long (Fig. 2C,D). The mouth pore of *Solenofilomorpha* sp. 2 is further posterior, at U20–24, and the pharynx is shorter, in specimens of 1.25 mm body length the anterior wall measuring about 60 μm and the posterior wall 100 μm (Fig. 2 E,F). Despite individual variation, this difference in pharynx length between the two species of *Solenofilomorpha* is consistent. There is no frontal mouth area present that is comparable to that of *Myopea* sp. and the sphincters at the mouth pore are less pronounced. The musculature of the anterior pharynx consists of a simple grid of peripheral longitudinal and outer circular muscles. The posteriormost part of the pharynx is surrounded by strong circular muscles, and thus an extensive, funnel-shaped posterior sphincter is present. In *Solenofilomorpha* sp. 1 this sphincter is composed of a single layer of about 5–7 strong circular muscle fibers, but in *Solenofilomorpha* sp. 2 the circular muscle fibers are packed two to four layers thick and form a pronounced muscular funnel where the pharynx fuses with the digestive parenchyma (Fig. 2F). The circular fibers of the posterior sphincter are adluminal. In both *Solenofilomorpha* species the longitudinal pharynx fibers clasp the sphincter funnel, and thus the relative position of circular and longitudinal muscles is reversed in the posteriormost pharynx region. There are no pharyngeal glands.

Ultrastructure of the Mouth and Pharyngeal Musculature

The pharyngeal muscle cells of *Myopea* sp. and the *Solenofilomorpha* species are band-shaped, with thin lateral and terminal processes. Most are about $1 \times 2 \mu\text{m}$ in diameter (Fig. 3A), but the large sphincter fibers may be up to four times as thick. Adjacent muscle fibers, both longitudinal and circular, are connected to each other by desmosome-like junctions (Fig. 3B). The sphincter fibers at the mouth and at the posterior end of the pharynx are especially strongly linked to longitudinal and radial fibers (Fig. 3C). In *Myopea* sp., the mouth sphincter fibers bear long lateral processes that run parallel to the numerous radial muscle fibers between the mouth epithelium and the body wall

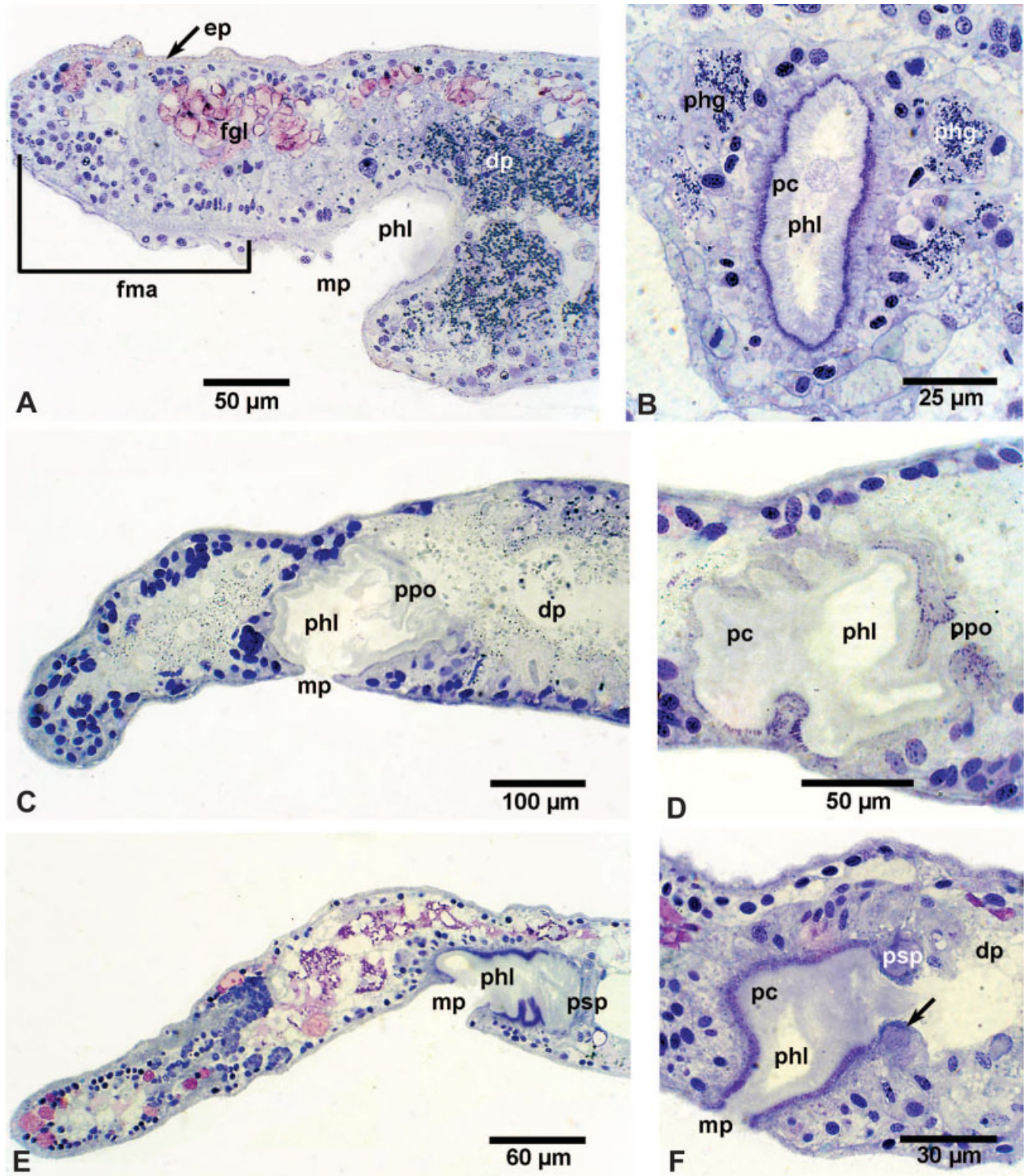


Fig. 2. Position and histology of the pharynx in *Myoecetes* sp. (A,B), *Solenofilomorpha* sp. 1 (C,D), and *Solenofilomorpha* sp. 2 (E,F). Semithin sections stained with Toluidine blue. A,C–F: Sagittal sections, anterior body tip to the left. Note differences among species in the location of the mouth pore (mp) in relation to the anterior body tip, the extensive frontal mouth area (fma) in *Myoecetes* sp. (A), and the darkly staining glandular cells in the posterior pharynx of *Solenofilomorpha* sp. 2 (arrow in F). B: Cross-section through the pharynx of *Myoecetes* sp. with pharyngeal glands (phg). dp, digestive parenchyma; ep, epidermis; fgl, frontal glands; mp, mouth pore; pc, pharynx cilia; phl, pharynx lumen; ppo, posterior pharynx opening; psp, posterior pharyngeal sphincter.

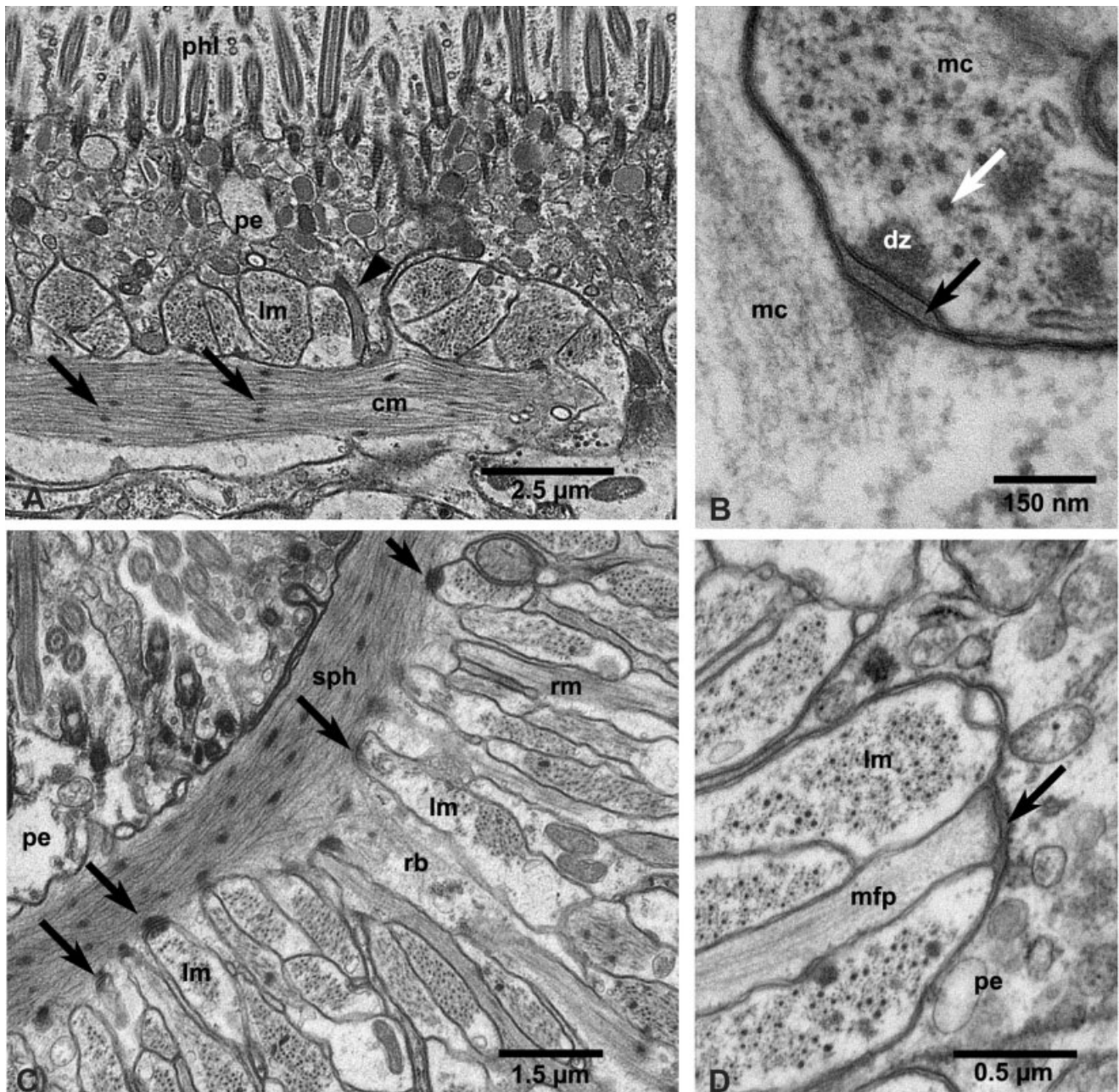


Fig. 3. Pharyngeal musculature of *Myopea* sp. TEM. **A:** Cross section of pharynx wall. Note the pseudo-striated arrangement of Z-bodies (arrows) in the circular muscle fiber (cm) and the lateral process (arrowhead) of one of the longitudinal muscle fibers (lm) fixed to the pharynx epithelium (pe). **B:** Desmosome-like junction between muscle cells (mc) with dense zone (dz) of accumulated fibrils and dense matrix material between the cell membranes (black arrow). The white arrow points out a thick myofilament in cross-section. **C:** Cross-section of mouth wall showing mouth sphincter fiber (sph) with radial process (rp) branching off. Note the desmosome-like junctions (arrows) between the sphincter fiber and a number of longitudinal muscle fibers (lm). **D:** Detail of a spot-like junction (arrow) between a process of a muscle fiber (mfp) and a cell of the pharyngeal epithelium (pe). lm, longitudinal muscle fiber; pe, pharyngeal epithelium; phl, pharynx lumen; rm, radial muscle fiber.

(Fig. 3C). Short lateral and terminal processes of both circular and longitudinal muscles turn toward the epithelium and are connected to epithelial cells by spot-like junctions (Fig. 3A,D). The orientation of myofilaments within these processes is perpendicular to that within the main

fiber (Fig. 3A). In the myofilament-containing portion of each muscle cell, there are 5–8 small myofilaments for each thick myofilament. The dense, rod-shaped Z-bodies are mostly irregularly scattered, but sometimes are arranged in rows that run obliquely across a muscle fiber (Fig. 3A).

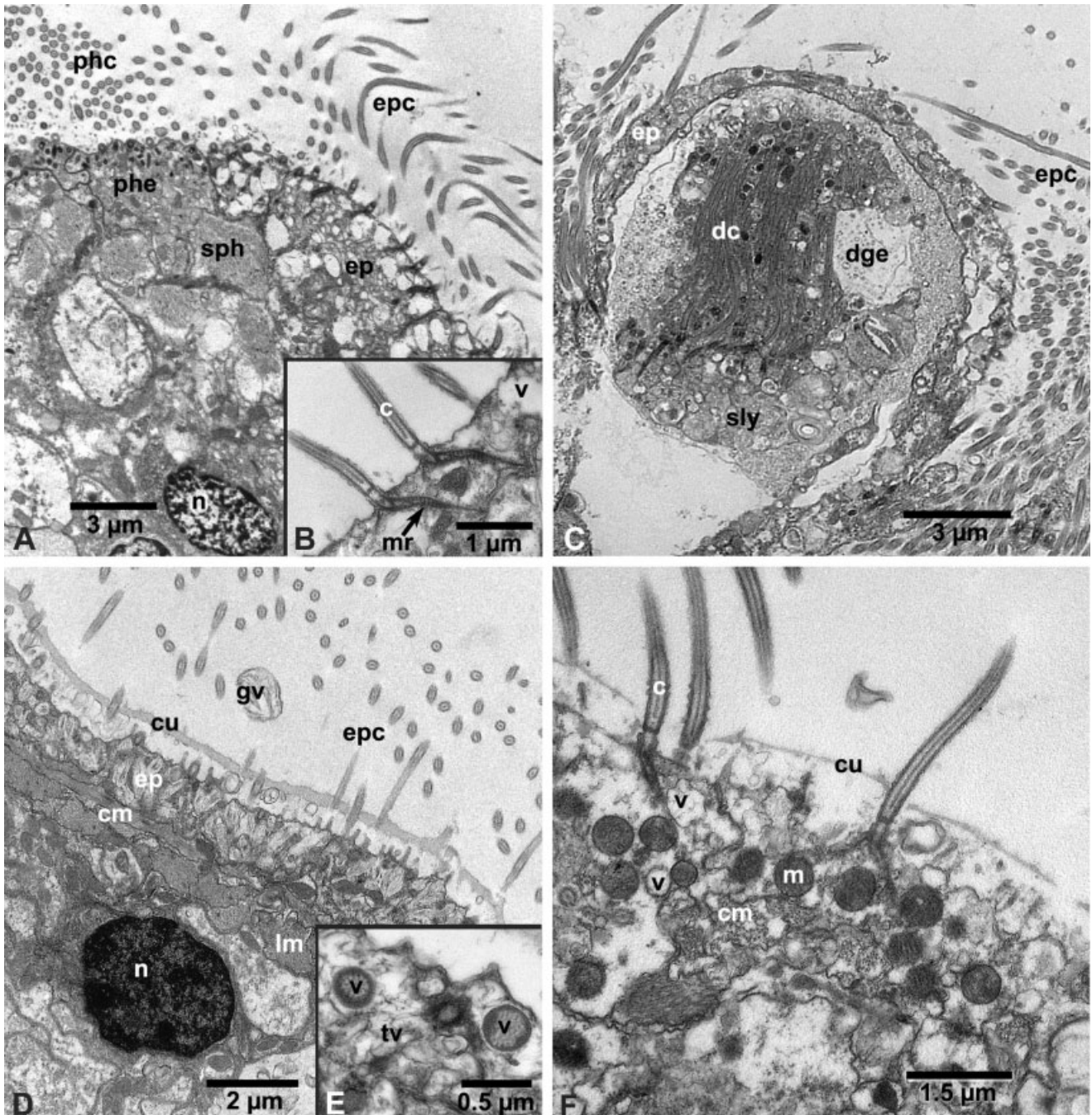


Fig. 4. Epidermis near the mouth. TEM. **A,B:** *Myopea* sp. **A:** Junction of pharynx epithelium (pe) to epidermis (ep) with mouth sphincter (sph) in cross section. Note the numerous transparent vesicles in the epidermis. **B:** Epidermal cilia (c) with bent main rootlets (mr). **C:** *Myopea* sp. Degenerating epidermal cell (dge) with decomposing cilia (dc) and secondary lysosomes (sly) sunk below an epidermal cell (ep). **D:** *Solenofilomorpha* sp. 1. Cross-section of the body wall. Note epidermis (ep) with transparent glandular vesicles, one of which is detached (gv), and long cilia (epc), covered by cuticle-like layer (cu). **E:** Detail of epidermal cell apex with transparent vesicles (tv) and specific layered vesicles (v). **F:** *Solenofilomorpha* sp. 2, longitudinal section. Note the thin cuticle-like layer (cu) pierced by cilia (c) and the high number of electron-dense mitochondria (m) and small transparent vesicles (v). cm, circular muscle fiber; epc, epidermal cilia; lm, longitudinal muscle fiber; n, nucleus of epidermal cell.

Epidermis Near the Mouth

In the species studied herein the epidermis surrounding the mouth is a secretory epithelium com-

posed of tall, multiciliated cells. Most of the cell somata are situated below the body wall musculature and connect to their apices by elongate necks (Fig. 4A). The apex of each epidermal cell is highly

TABLE 1. Cell and cilia dimensions and cilia density in the epidermis and pharynx of the studied species of solenofilomorphids

	<i>Myopea</i> sp.	<i>Solenofilomorpha</i> sp. 1	<i>Solenofilomorpha</i> sp. 2
Epidermis			
Cell height (μm)	16.0–22.0	10.0–15.0	10.0–15.0
Apex height (μm)	4.0–6.0	1.5–2.5	2.0–3.0
Apex width (μm)	12.0–16.0	12.0–15.0	12.0–15.0
Number of cilia/10 μm^2	30	40	36
Cilia length (μm)	8.0	6.0	6.2
Ciliary tip length (μm)	2.0	0.6	0.7
Main rootlet length (μm)	2.3	1.7	1.8
Mouth and pharynx			
Cell height (μm)	25.0–35.0	18.0–20.0	12.0–15.0
Apex height (μm)	3.0–5.0	2.0–3.0	2.0–3.0
Apex width (μm)	10.0–12.0	15.0–20.0	10.0–12.0
Number of cilia/10 μm^2	160	530	144
Cilia length (μm)	18.0	6.0	6.2 (10.0)
Ciliary tip length (μm)	1.0	0.4	0.4
Main rootlet length (μm)	2.3	2.0	2.0

broadened compared to the basal part of the cell. Details on the size of epidermal cells for each species are given in Table 1. Apically, all cells are connected through adherens junctions, but septate junctions and the apical cell web are indistinct. The apical cell membrane bears regularly set cilia interspersed with short microvilli. The cilia exhibit the characteristic morphology of acoel locomotory cilia (Fig. 4B). They are loosely spaced and the slender main rootlets point rostrally. Densities and dimensions of cilia in each species are shown in Table 1.

Degenerating epidermal cells occur regularly. They become dislocated from the epidermis and may be entirely sunk below it. They are globular and are characterized by a partly decomposed nucleus, internalized cilia showing different degrees of decomposition, numerous vesicles, and primary and secondary lysosomes (Fig. 4C).

***Myopea* sp.** The apices of epidermal cells contain large, amorphous vacuoles that evidently form by fusion of smaller secretory vesicles. These vesicles are filled with a secretion that is metachromatic with Toluidine blue, resulting in a pink coloration (Fig. 2A). In electron microscopy, the secretory material is transparent, with strands of fibrous material (Fig. 4A). All this is characteristic of glycosaminoglycans (GAGs). In most cells the apices are full of secretory vacuoles, and the cytoplasm, containing mitochondria and small transparent vesicles, is limited to narrow spaces around the ciliary rootlets and near the cell membrane. The apical microvilli are up to 1 μm long and often are branched. The epidermal cilia are arranged in rows (12–16 μm apart) of close-set cilia (6–9 μm apart).

***Solenofilomorpha* sp. 1.** The cell apices are packed with transparent mucous vesicles smaller than those of *Myopea* sp. (Fig. 4D). The most conspicuous difference, however, is the presence of a cuticle-like glycocalyx layer 0.4–0.5 μm thick that covers the entire epidermis of *Solenofilomorpha* sp. 1 (Fig. 4D). This layer is not adjacent to the apical

cell membrane but sits above it by about 1–1.3 μm , a distance equaling the average length of the microvilli. The microvilli are hardly ever branched and have electron-dense cores and dense tips. Like the microvilli, the epidermal cilia are sparsely set at spacings of 6–12 μm , but they pierce the cuticle. In addition to the large mucous vesicles with partly fibrous contents, the cell apex contains numerous small tubular transparent vesicles with a diameter of about 0.1 μm . The mucus does not appear to play a role in cuticle formation but it is secreted in big lumps (Fig. 4D), whereby the cuticle becomes locally ruptured. Some of the small, tubular vesicles, in contrast, occur in the gap between the apical cell membrane and the cuticle and fuse with the cuticle. Egg-shaped epitheliosomes, which are about 0.3 μm long and 0.2 μm wide, are especially numerous near the apical cell membrane. These epitheliosomes have a dense margin and a more transparent core, with a coarsely fibrous substructure (Fig. 4E).

***Solenofilomorpha* sp. 2.** The epidermis is similar to that of *Solenofilomorpha* sp. 1 in most cytological features. The cuticle-like layer, however, is considerably thinner (about 0.2 μm) and the apical microvilli are even more sparsely set and shorter, up to 0.7 μm in length (Fig. 4F). The epidermal cells contain numerous transparent vesicles with flocculent or granular dense material. These vesicles are irregular in shape and measure 0.3–0.8 μm in section. Many small, roughly globular vesicles with a diameter of 0.1–0.3 μm and transparent contents are present between the apical membrane and the cuticle-like layer. There are no electron-dense epitheliosomes in the epidermal cells but a large number of mitochondria, are located mostly between or directly below the ciliary rootlets (Fig. 4F).

Epithelium of the Mouth and Pharynx

Multiciliated epithelial cells interspersed with monociliated receptor cells and unicellular pharynx-

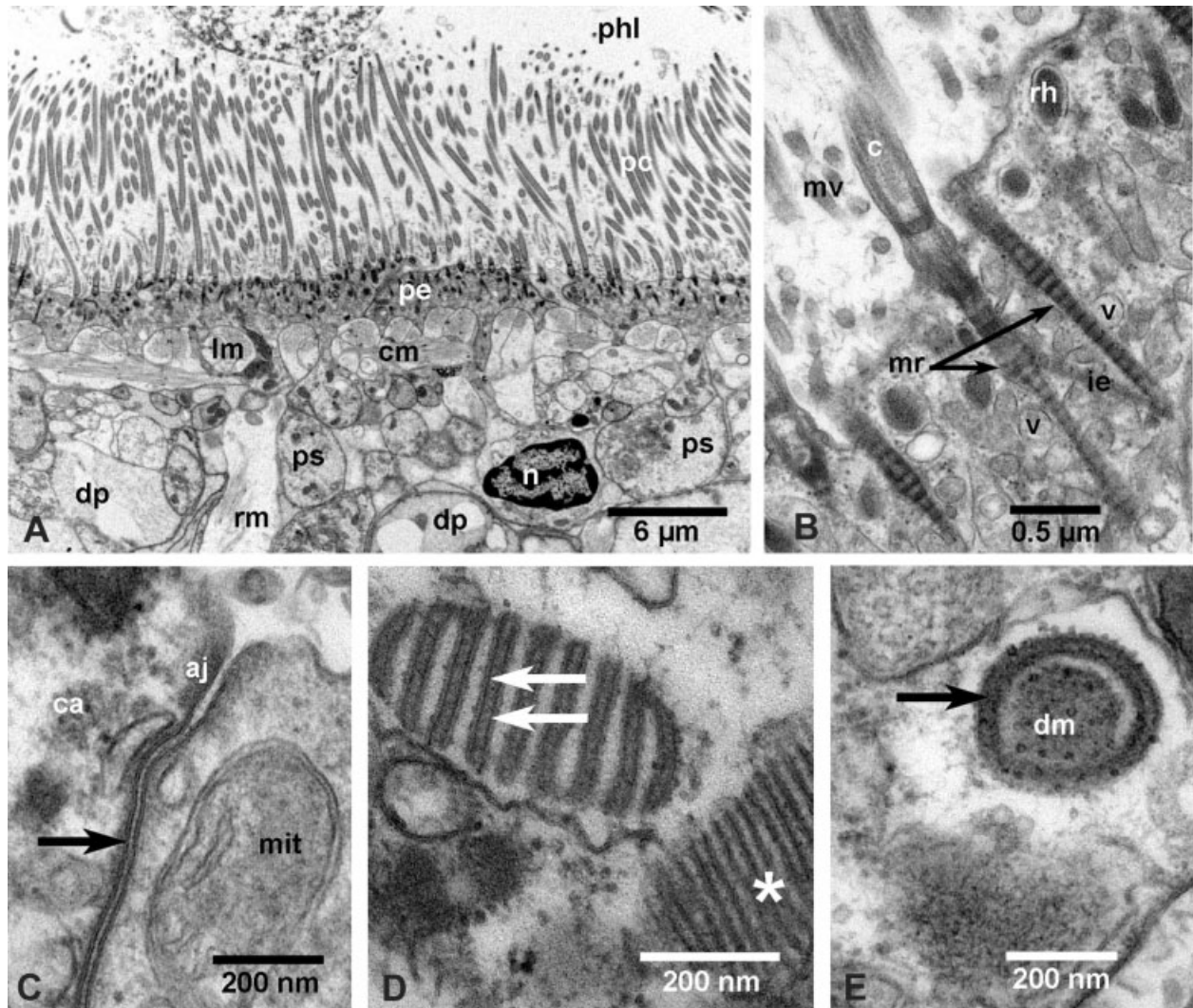


Fig. 5. Mouth and pharynx epithelium of *Myopea* sp. TEM. **A:** Cross-section through the pharynx wall composed of pharyngeal epithelium (pe) bearing long cilia (pc) and insunk somata (ps), and of longitudinal (lm) and circular muscles (cm). **B:** Pharyngeal cilium (c) with long main-rootlets (mr) and striated interconnecting element (ie) fixed to neighboring main rootlet. Note transparent vesicles (v) and dense epitheliosomes (rh). **C:** Connection between cell apices (ca) of the pharyngeal epithelium; apical adherens junction (aj) followed by region resembling septate junction but lacking distinct septa (arrow). **D:** Lamellar bodies, stacks of cisternae in longitudinal section showing double membranes (arrows); note the dense packing of stacks in one of the bodies (asterisk) compared to the other one. **E:** Cup-shaped cisterna of lamellar body showing tangential section of membrane (dm) surrounded by double membrane of the rim (arrow). dp, digestive parenchyma; mit, mitochondrion; mv, microvilli; rm, radial muscle.

geal glands line the mouth and pharynx. Receptors and pharyngeal glands are described below. In the mouth and pharyngeal epithelium, as in the epidermis, adjacent cells are connected through apical adherens junctions, but apical cell web and septate junctions are indistinct (Fig. 5C).

The apices of the multiciliated epithelial cells are flat but highly broadened; the cell neck passing through the musculature of the mouth or pharynx is of variable diameter. For measurements, see Table 1. In the posterior pharynx, where the muscle layers are strong and thick, the cell necks may be narrow and highly constricted by the musculature. In some cells, however, there is no neck and the whole cell,

including the nucleus, is located distal to the muscle layers. Generally, the epithelial cells do not bear mucous vesicles like those of epidermal cells, but the cell apices contain a variety of epitheliosomes, and thus the cells also seem to have secretory functions. The apical cell surface bears microvilli scattered between the densely set cilia. These cilia are of the same general type as the epidermal cilia (Figs. 5B,C, 6C,D, 7C–E). In a continuation of the orientation of the main ciliary rootlets of epidermal cilia, rootlets in the dorsal part of the mouth and pharynx point obliquely rostrally. Ventral epidermal and pharyngeal ciliary rootlets also point obliquely rostrally, which is only possible due to a change in the relative

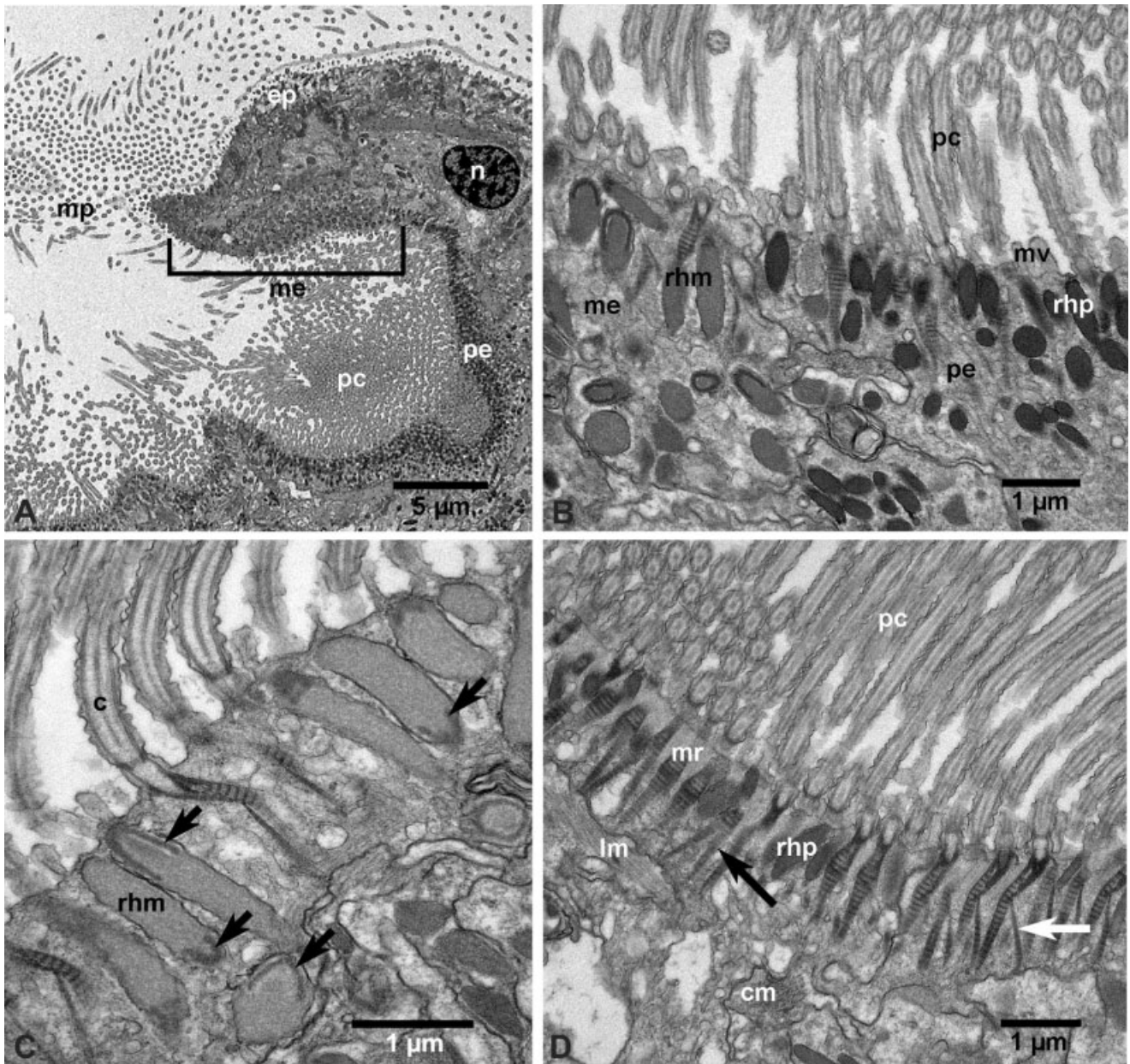


Fig. 6. Mouth and pharynx of *Solenofilomorpha* sp. 1. TEM. **A:** Oblique frontal section of part of the mouth and anterior pharynx; note the extension of the specialized mouth epithelium (me), which appears less dense than the pharyngeal epithelium (pe). **B:** Junction of mouth cells (me) with pharyngeal cells (pe) characterized by special dense epitheliosomes (rhm, rhp), respectively; note the short microvilli (mv). **C:** Detail of mouth cell apex with densely packed epitheliosomes (rhm) and cilia (c); note the cap-like dense areas of epitheliosomes (arrows). **D:** Apical part of pharynx epithelium with characteristic epitheliosomes (rhp) and dense ciliation; ciliary main rootlets (mr) partly in frontal section showing the broad side of the strap-shaped interconnecting elements (black arrow) and partly in sagittal section (white arrow). cm, circular muscle fiber; lm, longitudinal muscle fiber; pc, pharyngeal cilia.

orientation of rootlets at the junction of epidermis and pharyngeal epithelium (Fig. 7B).

Degenerating epithelial cells that are similar to degenerating epidermal cells occur sporadically in the mouth and pharyngeal epithelia.

Myopea sp. There is no difference in morphology of epithelial cells lining the mouth area and those of the pharynx proper. The pharyngeal cilia are over twice as long as the epidermal cilia but their nar-

rowed tips are shorter (see Table 1). The density of cilia is considerably higher than in the epidermis, with an average distance of 3–9 μm between neighboring cilia. The apical microvilli are partly branched. Beneath the apical membrane the epithelial cells are packed (Fig. 5A) with a variety of epitheliosomes (Fig. 5B,D,E). Three main types may be defined: dense epitheliosomes, medium-dense vesicles, and roughly globular transparent vesicles. The

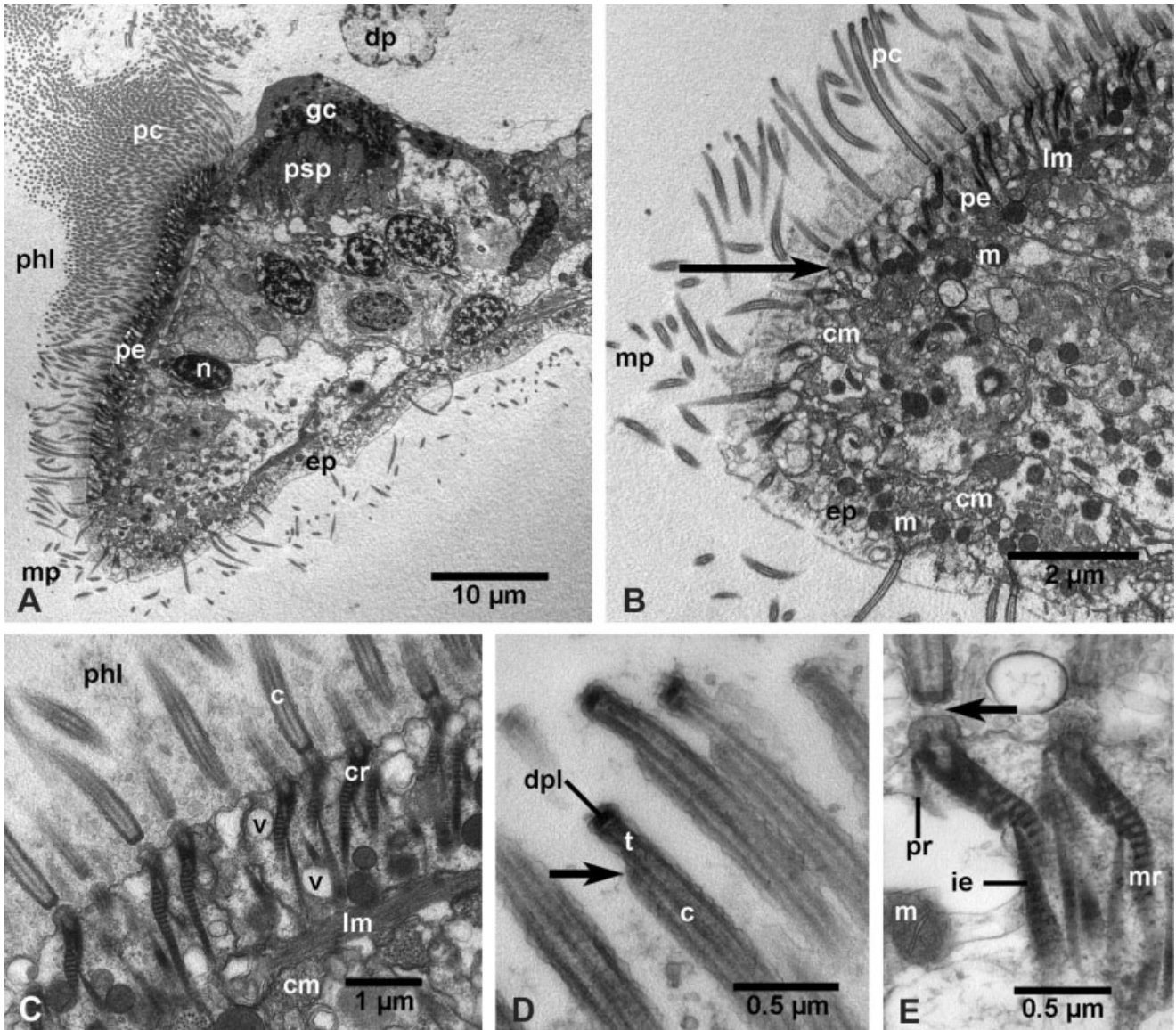


Fig. 7. Mouth and pharynx of *Solenofilomorpha* sp. 2. TEM. **A:** Overview of posterior pharynx wall and the adjoining ventral body wall in sagittal section; note the conspicuous difference in density of pharyngeal cilia (pc) and cilia of the epidermis (ep); the posterior pharyngeal sphincter (psp) is wrapped in glandular cells (gc). **B:** Detail of the ventral part of the mouth showing the change in orientation of ciliary main rootlets at the junction (arrow) of the pharyngeal epithelium (pe) with the ventral epidermis (ep); note the dense globular mitochondria (m). **C:** Detail of pharyngeal cell apex with closely set ciliary rootlets (cr) and transparent vesicles (v). **D:** Tips of pharyngeal cilia (c) with shelf-like narrowing (arrow), narrowed tip (t), and apical dense plate (dpl). **E:** Ciliary rootlet system of pharyngeal cilia with short posterior rootlets (pr), bent main rootlets (mr), and broad interconnecting elements (ie); note the gap between the ciliary basal body and the ciliary shaft (arrow) caused by a deep infolding of the ciliary membrane. cm, circular muscle fiber; dp, digestive parenchyma; lm, longitudinal muscle fiber; m, mitochondrion; mp, mouth pore; pe, pharyngeal epithelium; phl, pharyngeal lumen.

dense epitheliosomes are rod-shaped organelles, up to 1.2 μm long and 0.5 μm wide, and have a cross-striated substructure with a periodicity of 7 nm. The medium-dense vesicles are up to 0.6 μm long and about 0.4 μm in diameter. Their content is a finely granular substance, sometimes with additional dense granules. This type of vesicle is rare in comparison to the dense epitheliosomes, and even more so in comparison to the transparent vesicles. The

size of transparent vesicles varies to a great extent, with a maximum diameter of about 0.8 μm . Vesicles with internalized smaller vesicles are often present and many vesicles contain small amounts of dense granular or flocculent material. In the cell apex there are also conspicuous bodies that are up to 0.7 μm long and 0.4 μm wide and that seem to be composed of ring-shaped stacks of double membranes or cisternae (Fig. 5D,E). The central cavity of the

these cisternae is filled with a medium-dense matrix. The distance between the cisternae varies considerably, whereby in poorly fixed specimens they are distinctly separated and no central matrix is present.

***Solenofilomorpha* sp. 1.** Surrounding the mouth opening are 3–4 rings of cells that differ from the epithelial cells of the pharynx proper in the kinds of epitheliosomes they bear (Fig. 6A,B). The most peripheral of these mouth cells are partially covered by the cuticle characteristic of the epidermal cells, and which seems to stem from the adjacent epidermal cells rather than from the mouth cells themselves. The cells are tall and somewhat irregularly shaped, with broad cell necks. The cilia are the same length as epidermal cilia, but their narrowed tips are shorter (see Table 1). The density of cilia is more than 10 times higher than in the epidermis, with distances of no more than 2–6 μm between cilia, and the interconnection between the ciliary rootlets is strong, with broad, strap-shaped interconnecting elements. The mouth cells bear relatively large, medium-dense epitheliosomes, measuring up to 1.5 μm in length and 0.4–0.5 μm in width. In many of these epitheliosomes, parts of the near-marginal zone are denser than the core and the margin proper (Fig. 6C). The density of epitheliosomes is highest in the cell apex near the apical cell membrane. Transparent vesicles that are irregular in shape, about 0.1–0.3 μm in size, and contain flocculent material that is scattered within the cytoplasm of the entire cell, but are most numerous in the apex.

The epithelial cells of the pharynx proper are characterized by smaller, denser epitheliosomes that are up to 1 μm long and about 0.4 μm wide (Fig. 6B). The density of these epitheliosomes again is highest between the ciliary rootlets, but mature dense epitheliosomes also occur in the cell neck and cell body, which gives the cells a glandular look. Apart from the size and electron density of epitheliosomes, the morphology of pharyngeal cells is similar to that of the mouth cells, with densely set cilia having broad interconnecting rootlets (Fig. 6D).

***Solenofilomorpha* sp. 2.** The pharyngeal epithelium directly adjoins the epidermis (Fig. 7A,B) and is slightly thinner than that of *Solenofilomorpha* sp. 1. The overall appearance of the pharyngeal epithelium is quite similar to that of the epidermis, but the pharyngeal cilia are 4–5 times more densely set (Fig. 7C; see Table 1). The length of cilia is similar in the pharynx and epidermis, except for a ring-shaped zone of posterior pharynx cells at the opening into the digestive parenchyma, where the cilia are almost twice as long (10 μm). The narrowed ciliary tip is short and has the characteristic terminal dense plate of acoel cilia (Fig. 7D). There is a deep infolding of the membrane surrounding the basal body, shortly below the basal plate (Fig. 7C,E). The posterior ciliary rootlet is thin but the lateral interconnecting elements of the long and slender main rootlets fan out broadly (Fig. 7E).

The cell apices are full of transparent vesicles, partly with flocculent contents, which are globular to egg-shaped and measure 0.4–0.6 μm in diameter (Fig. 7C). These vesicles are frequently secreted into the pharynx. Smaller vesicles, 0.05–0.1 μm in diameter, are numerous in the cell apices and the somata. There are no dense epitheliosomes in the pharyngeal epithelium of *Solenofilomorpha* sp. 2. This lack of dense bodies accentuates the high density of mitochondria near the cell apex and between the ciliary rootlets (Fig. 7B).

Receptor Cells

Receptor cells of the mouth and pharynx are very sparsely set between the epithelial cells. The apex of receptor cells bears some long microvilli, but these do not form a collar around the single cilium, which is at the same level as the cilia of adjacent epithelial cells. The large main rootlet of each receptor cilium is spindle-shaped but slightly or distinctly curved, depending on the species. The cytoplasm of the apical portion of the receptor dendrite contains some mitochondria, numerous small transparent vesicles, and a few dense vesicles with a diameter of about 0.1 μm . The dendrite passes through the muscle layers and the receptor-cell soma is located proximal to the mouth and pharyngeal musculature.

***Myopea* sp.** The density of receptor cells is highest in the mouth region, but even there no more than a single receptor was present in each ultrathin cross-section. The main ciliary rootlet is straight, more than 2 μm long and up to 1.3 μm broad (Fig. 8A), and has a pointed tip (Fig. 8B). The basal body has a distinct dense plate with a lamellar region beneath (Fig. 8C).

***Solenofilomorpha* sp. 1.** The receptor cells are restricted to the region near the mouth—thus neighboring cells may be epidermal cells, mouth cells, or pharyngeal cells. The main rootlet of the receptor cilium is about 2 μm long and 0.8 μm wide. It is more curved and less compact (Fig. 8D) than that in receptor cells of *Myopea* sp. There is a short posterior rootlet that in the posterior pharynx wall points in the opposite direction compared to the posterior rootlets of the surrounding locomotory cilia (Fig. 8E). Thus, the receptor does not seem to take part in the change of orientation of locomotory cilia mentioned above.

***Solenofilomorpha* sp. 2.** Receptor cells could not be observed in this species. Their total absence is improbable but they must be sparsely set, if present.

Pharyngeal Gland Cells

Only in *Myopea* sp. are there tall unicellular pharyngeal glands opening individually into the anteriormost portion of the pharynx. Most glands are located along the lateral pharynx walls. Each

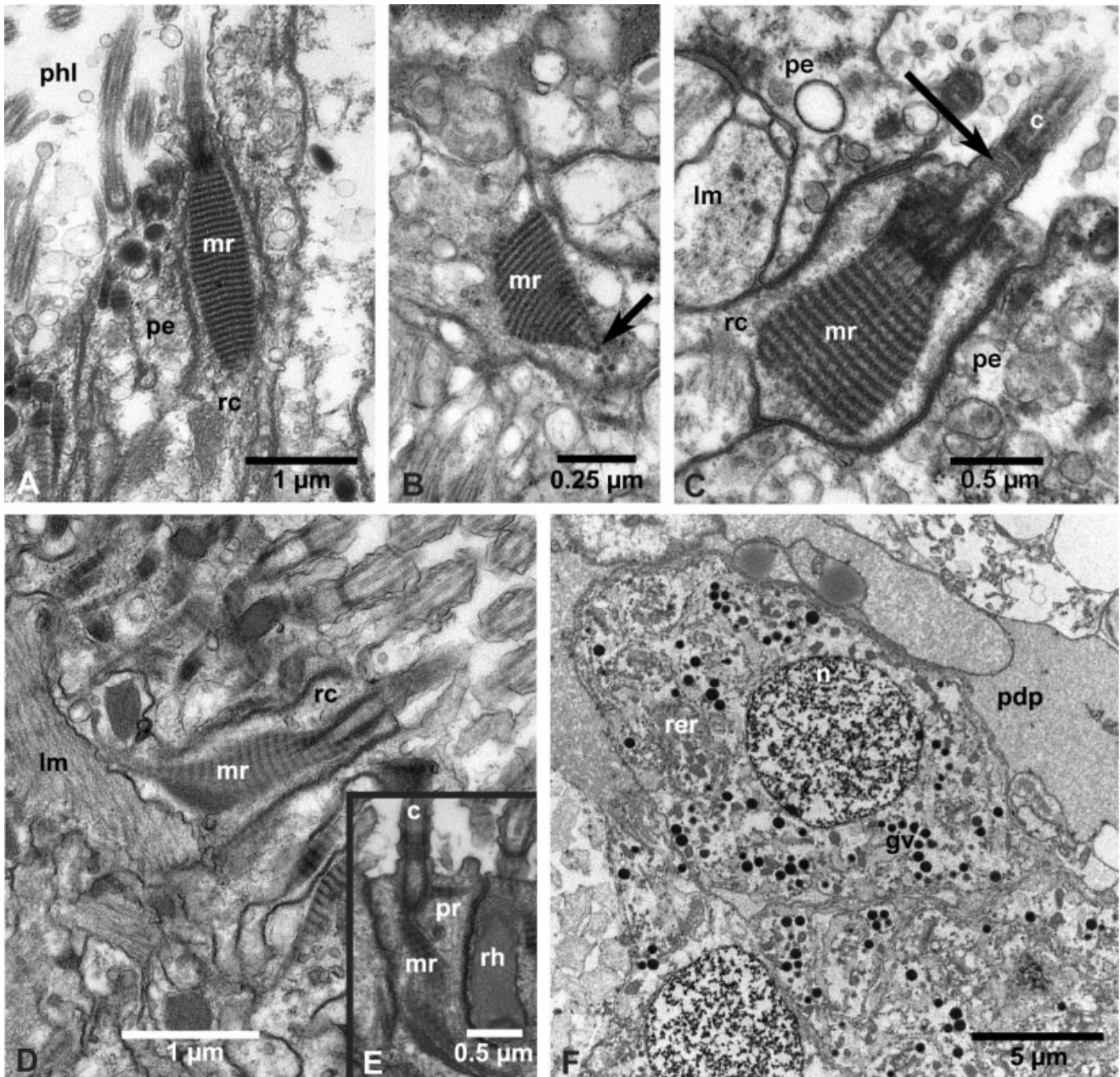


Fig. 8. Receptor cells of the mouth and pharynx (A–E) and pharyngeal glands (F). TEM. A–C: *Myopea* sp. A: Apex of receptor-cell (rc) bearing single cilium with large main rootlet (mr), surrounded by epithelial cells (pe). B: Pointed tip (arrow) of the main rootlet of a receptor cilium (mr). C: Detail of basal body and part of the main rootlet (mr) of the cilium (c) surrounded by pharynx epithelium (pe); note the lamellar substructure in the basal body (arrow). D, E: *Solenofilomorpha* sp. 1, receptor cells (rc) with single cilium (c) bearing large, curved main rootlet (mr); note the short but strong posterior rootlet (pr) in E. F: Pharynx glands of *Myopea* sp. exhibit insunk cell bodies with considerable rough endoplasmic reticulum (rer), small glandular vesicles (gv), and large nuclei (n) surrounded by cells of the peripheral digestive parenchyma (pdp). lm, longitudinal musculature; phl, pharyngeal lumen; rh, epitheliosome.

gland cell has a narrow and inconspicuous apex, a long neck passing through the pharynx musculature, and a wide soma that contains the large nucleus, plenty of rough endoplasmic reticulum, many free polyribosomes and dictyosomes, and globular to egg-shaped glandular vesicles (Fig. 8F). These vesicles measure between 0.4 and 0.7

μm in diameter, stain dark blue with Toluidine blue (Fig. 2B), and are uniformly dense in TEM. Some mitochondria are scattered in the soma and the proximal part of the cell neck. The cell neck also contains vesicles. In all specimens the number of vesicles within the cell necks decreased toward the pharynx lumen.

Cells Adjoining the Pharynx Epithelium and Digestive Parenchyma

The digestive parenchyma of the investigated species consists of large cells that are irregular in shape with long, tapering lateral processes. These cells obviously undergo considerable changes depending on the feeding status of the respective specimen. Gut contents generally were reduced to small pieces but regularly, at least in part, were composed of green plant material. A detailed analysis of the digestive parenchyma, however, is not within the scope of this study and thus the results given here are restricted to characteristics of the areas near the pharynx. These results can be applied to the digestive parenchyma as a whole only with reservations.

In the species investigated herein there is a ring-shaped zone of glandular cells at the junction between the pharynx epithelium and digestive parenchyma. The surface of these cells is free of cilia and microvilli. The digestive parenchyma consists of a central portion that either encloses a central lumen or, in some specimens, is fused to a (partial) central syncytium, and of a peripheral portion of so-called wrapping cells where no signs of syncytiality are evident.

***Myopea* sp.** Adjacent to the pharyngeal epithelium is a single row of large and partially flattened cells with high numbers of transparent, globular vesicles (Fig. 9A). The vesicles are of variable size, ranging from 0.2–1 μm in diameter. Most vesicles contain some fibrous or granular dense material, and many large vesicles contain smaller ones (Fig. 9B). Vesicles are also attached to the free cell membranes toward the central lumen and similar vesicles occur within the lumen. The nucleus lies centrally within each cell and the posterior sphincter fibers are covered and partially wrapped by these glandular cells (Fig. 9A). No distinct cell junctions could be detected between these cells or between them and adjacent pharyngeal and digestive parenchyma cells.

Cells of the central parenchyma stain lightly with Toluidine blue and they either constitute a thin layer of cells surrounding a central lumen (Fig. 9C) or the central lumen is displaced by a central syncytium. In any case, the cells have long processes projecting outwards between the cells of the peripheral parenchyma. They contain transparent vesicles and secondary lysosomes. The peripheral parenchyma is thick and much more conspicuous even in light microscopy (Fig. 2A). The cells are arranged in multiple layers and are characterized by a uniformly staining, medium-dense cytoplasm and by numerous large lipid droplets measuring up to 3 μm in diameter (Fig. 9C). In the vicinity of the lobed nucleus are clusters of smooth endoplasmic reticulum. Mitochondria and strands of fibrous material are evenly scattered through the cell, but vacuoles and occasional membrane whorls are restricted to small

regions close to the cell margin. The pharynx can be retracted partially into the peripheral digestive parenchyma (Fig. 2A).

***Solenofilomorpha* sp. 1.** The specialized cells adjacent to the pharynx are similar to those of *Myopea* sp., with transparent cytoplasm and numerous transparent vesicles varying in size from 0.2–0.6 μm (Fig. 9D). Differences between the central and peripheral parenchyma are not as distinct as in *Myopea* sp.

All parenchymal cells are large, irregular in shape, and have a light cytoplasm. The central cells are predominantly characterized by transparent vesicles and large secondary lysosomes (Fig. 9E). Additionally, they contain scattered lipid droplets measuring 0.4–0.8 μm in diameter. In the peripheral cells, the cytoplasm also is transparent. There are no secondary lysosomes but their cytoplasm contains numerous transparent vesicles of varying sizes, high numbers of dense mitochondria, and medium-dense lipid droplets with a diameter of 1.5–2 μm (Fig. 9F).

***Solenofilomorpha* sp. 2.** There is a single row of conspicuous glandular cells between the pharyngeal epithelium and the central digestive parenchyma, thus in the same location as the specialized cells with transparent vesicles in *Myopea* sp. and *Solenofilomorpha* sp. 1 (Fig. 9G). These cells contain dense elongate vesicles that are up to 1.5 μm long and 0.6 μm wide (Fig. 9H). Additionally, there are numerous transparent vesicles, some containing internalized smaller vesicles, measuring 0.3–0.8 μm . The cytoplasm of these cells is dense. The structure of the central and peripheral digestive parenchyma is similar to that of *Solenofilomorpha* sp. 1.

DISCUSSION Pharyngeal Characters of Solenofilomorphidae

In accordance with Crezée's definition of the solenofilomorphid pharynx (Crezée, 1975; see also Doe, 1981), the pharynges of the three species investigated herein show a number of common features. First and perhaps most important, the relationship between their body wall muscles and their pharyngeal muscles is unique among acoels in that all longitudinal body wall muscles bypass the mouth, while specialized circular muscles run into the pharynx to form at least part of the longitudinal pharyngeal musculature. When contracting, these muscles may act to widen the mouth opening and at the same time protruding the pharynx toward or even through the mouth. A similar configuration with longitudinal muscles bypassing the mouth is found in the pharynx simplex of the catenulid *Stenostomum leucops* (see Hooze, 2001) and the pharynx simplex coronatus of Macrostomida and Haplopharyngida (Doe, 1981). This similarity, however, has to be interpreted as an analogy because of striking

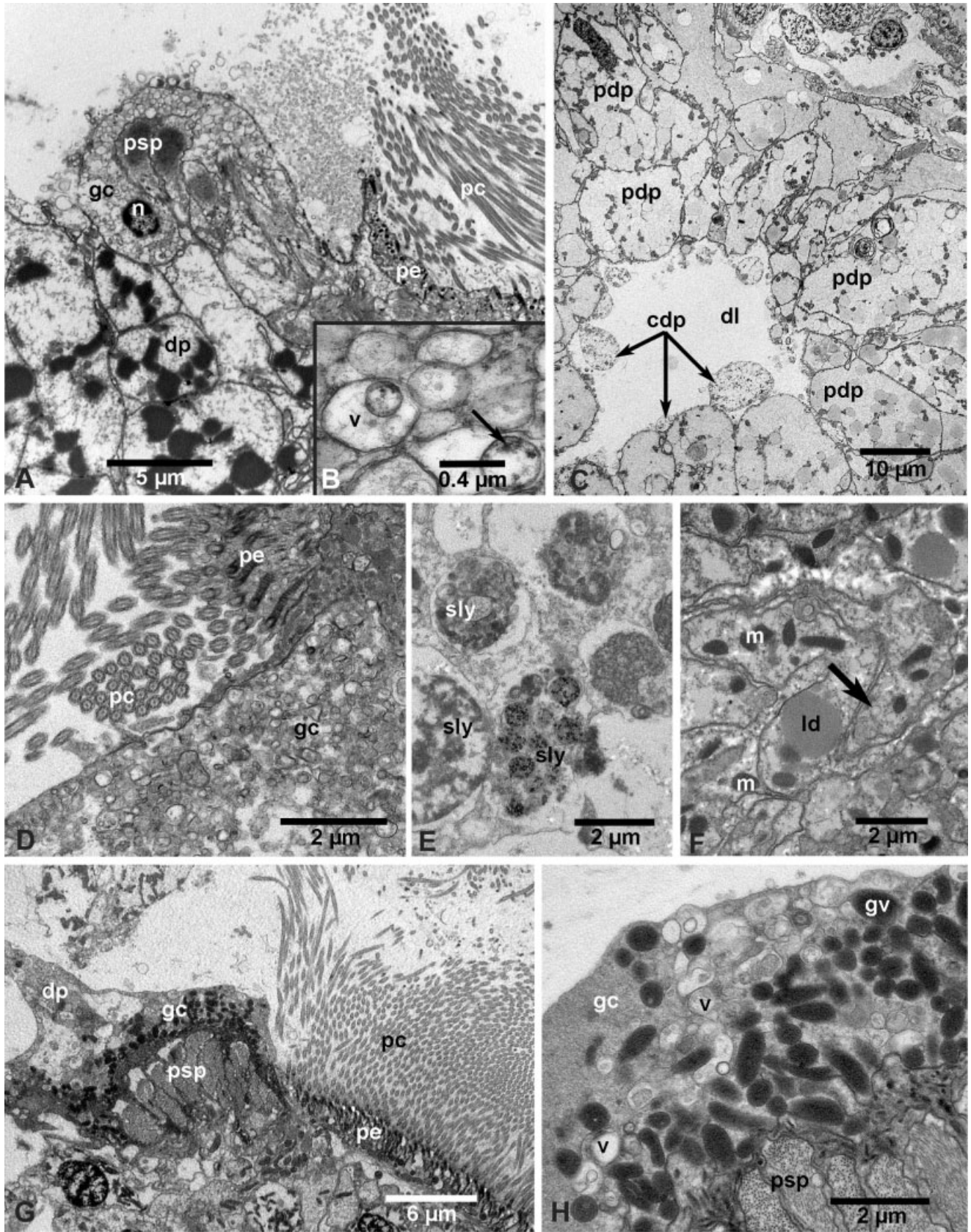


Figure 9

differences in other pharyngeal characters and considering the phylogenetic distance between these taxa. Significantly, the relative position of circular and longitudinal muscle layers in the anterior pharynx of our specimens is the reverse of that described by Crezée (1975) and Doe (1981). Only in the posteriormost part of the pharynx of our *Solenofilomorpha* species, specifically at the funnel-like posterior sphincter, are the circular muscle fibers adluminal.

The pharyngeal epithelium of solenofilomorphid species generally is secretory and ciliated, with the cilia more closely spaced than in epidermal cells. Like Doe (1981), we confirm Crezée's (1975) observation that the pharyngeal cilia are generally of the same structure and thickness as the epidermal cilia, even if their relative length varies between the genera (see below).

Glandular cells surrounding the posteriormost part of the foregut were not mentioned in previous reports on Solenofilomorphidae, most likely because, as in *Myopea* sp. and *Solenofilomorpha* sp. 2, they may be inconspicuous. In a number of other acoel taxa, however, such a nonciliated esophageal region between the ciliated pharynx and the digestive parenchyma is known and sometimes is much more pronounced than in the studied species of solenofilomorphids. It may be lined by eosinophilic glandular cells, as in *Hofstenia atroviridis* (see Bock, 1923), and *Hofsteniola pardii* (see Papi, 1957), or by spongy cells with many vesicles, as in *Oligochoerus limnophilus* (see Ax and Dörjes, 1966), or described to be coated by mucus (Riedl, 1954, for *Nadina pulchella*). In diopisthoporids, in contrast, the nonciliated part of the pharynx is lined by a microvillar epithelium (Doe, 1981, for *Diopisthoporus* sp.; Smith and Tyler, 1985, for *D. gymnopharyngeus*), which may have additional secretory functions (Westblad, 1940,

for *D. longitubus*). No ultrastructural data are available for comparison.

Ultrastructural features shared by the species investigated herein include scattered monociliated receptor cells, the receptor cilium with a leaf-shaped main rootlet. For *Solenofilomorpha funilis*, details on the sensory cilia are lacking but receptor cells "which appear to be monociliated" occur along the lateral walls of the mouth (Doe, 1981).

A pseudo-striation of muscles, as found in the pharyngeal muscles of the species examined in this study, is characteristic for fast musculature. Most turbellarians, including the acoels, have predominantly smooth musculature, although an oblique striation occasionally has been reported (Tyler, 1984; Rieger et al., 1991). There is no detailed information, however, on the ultrastructure of the pharyngeal musculature of other pharynx-bearing acoel taxa, and thus the phylogenetic value of this feature remains unknown.

Genus-Specific Characters

Some characters differ considerably between species of *Myopea* and *Solenofilomorpha*. Most conspicuously, the position of the mouth in all known *Myopea* species is more anterior, while, in general, the mouth in *Solenofilomorpha* is located more posteriorly. Other solenofilomorphid genera show intermediate positions (Crezée, 1975).

Long-necked unicellular pharyngeal glands occur exclusively in *Myopea*. Similar glands were reported for another solenofilomorphid, *Endocincta* (Crezée, 1975). Doe (1981) describes gland cell necks opening into the pharynx of *Solenofilomorpha funilis* but he did not detect any gland cell bodies in his TEM study and he did not encounter pharyngeal glands in histological preparations. Serous pharyngeal glands similar to those in *Myopea* are reported to produce protein-rich, often enzymatically active secretions in various invertebrates (e.g., Michel, 1988; Westermann and Schipp, 1998).

Pharyngeal cilia of the investigated species are more densely set than are epidermal cilia. While in *Myopea* sp. pharyngeal cilia are distinctly longer than epidermal cilia, in *Solenofilomorpha* pharyngeal and epidermal cilia are of about the same length, except for those in a ring-shaped zone in the posteriormost pharyngeal region, where they are longer.

The epidermis of *Solenofilomorpha* species produces a cuticle-like layer near the tips of microvilli (Doe, 1981; Rieger, 1984; and this study). Such a layer is absent in specimens of *Myopea* sp. and was not found in TEM preparations of the solenofilomorphid *Endocincta punctata* (Crezée, 1975). The layer is detectable in histological sections of well-fixed specimens, but as Crezée (1975) did not mention it for *Solenofilomorpha*, it may also have been over-

Fig. 9. Glandular cells at the junction between pharynx and digestive parenchyma and digestive parenchyma. TEM. **A–C:** *Myopea* sp. **A:** Posterior pharynx in sagittal section with glandular cells containing transparent vesicles (gc) wrapped around the posterior pharynx sphincter (psp); note dense lipid droplets within the peripheral digestive parenchyma cells (dp). **B:** Detail of glandular cell of the posterior pharynx showing transparent vesicles with incorporated smaller vesicles (v) and dense material (arrow). **C:** Digestive parenchyma near the pharynx in cross-section showing prominent peripheral digestive cells (pdp) and fragmented central digestive cells (cdp) that surround the distinct digestive lumen (dl). **D–F:** *Solenofilomorpha* sp. 1. **D:** Junction between pharynx epithelium (pe) and nonciliated glandular cell (gc). **E:** Detail of central digestive parenchyma cell with secondary lysosomes (sly). **F:** Peripheral digestive parenchyma cells with dense mitochondria (m), large lipid droplets (ld), and numerous transparent vesicles (arrow). **G,H:** *Solenofilomorpha* sp. 2. **G:** Junction between glandular cells (gc) covering posterior pharynx sphincter (psp) and pharynx epithelium (pe) with long cilia (pc). **H:** Glandular cell of posterior pharynx (gc) bearing numerous dense glandular vesicles (gv) and transparent vesicles (v). n, nucleus of glandular cell; pc, pharyngeal cilia; psp, posterior pharyngeal sphincter.

looked in the solenofilomorphid genera *Oligofilomorpha* or *Fusantrum*.

Species-Specific Characters

Certain pharyngeal characters may be tied to variations in feeding habits and prey, although we were not able to identify gut contents. These characters include the size of pharynx and probably specific features of the pharyngeal musculature. The strong, funnel-shaped posterior pharyngeal sphincter of *Solenofilomorpha* sp. 2 most likely can be projected through the mouth opening and serves for grabbing or even crushing food items, and then pulling them into the mouth. In *Solenofilomorpha* sp. 1 no such powerful sphincter is present, and the pharyngeal musculature is relatively weak, while in *Myopea* sp. the whole posterior pharynx is strongly muscular and funnel-like.

Epidermis and pharyngeal epithelium of the studied species are secretory and gain their specific aspect from a variety of epitheliosomes (see also Rieger et al., 1991). Most conspicuous are glycosaminoglycan (GAG) vesicles, which stain metachromatically with Toluidine blue and are electron transparent in TEM, and protein-rich serous vesicles that stain orthochromatically with Toluidine blue and are electron dense in TEM. Epidermal cells contain GAG-vesicles that are largest and most conspicuous in *Myopea* sp., while *Solenofilomorpha* sp. 1 in addition bears epitheliosomes with a core of filamentous material. For *Solenofilomorpha funilis*, in contrast, no GAG-vesicles but "small, dense granules that resemble dense ultrarhabdites" are reported for the epidermis (Doe, 1981). Mouth cells with epitheliosomes differing from those of the pharyngeal cells, as in *Solenofilomorpha* sp. 1, were not encountered in any other species of the genus, including *S. funilis*, where the ultrastructure of the mouth region was studied in detail (Doe, 1981). Specialized cells at the mouth that constitute a so-called transition zone between the epidermis and the pharyngeal epithelium were defined for Catenulida, Haplopharyngida, and Macrostomida (Doe, 1981). These cells are similar to epidermal cells but are insunk and with a different orientation of ciliary rootlets. Additionally, the transition zone is rich in receptor cells and gland-cell openings. A homology of the mouth region in *Solenofilomorpha* sp. 1 to this transition zone can be rejected based on differences in these characters as well on the isolated occurrence of specialized mouth cells within the genus *Solenofilomorpha*. Thus, we interpret the presence of mouth cells in *Solenofilomorpha* sp. 1 as a species-specific character. In pharyngeal cells of the investigated species the diversity of epitheliosomes is especially high. The density of epitheliosomes in pharyngeal cells varies within and between specimens, but nevertheless the respective assembly of epitheliosomes is species-specific. Thereby, electron-transparent vesicles

are always present and indicate general secretory activity. Electron-dense epitheliosomes similar to those in *Myopea* sp. and *Solenofilomorpha* sp. 1 were found as well in *S. funilis* (Doe, 1981) but are absent in *Solenofilomorpha* sp. 2. Additional layered or striated epitheliosomes occur in *Myopea* sp. and in *Solenofilomorpha* sp. 1.

Common Features

A number of features we encountered have been described similarly for representatives of other acoel taxa. We mention them here to highlight exceptions within the Acoela and among other flatworms.

The ciliated pharyngeal epithelium, if present, is insunk in most acoels, with the exception of *Hofstenia atroviridis* (Bock, 1923) and *H. miamia* (Steinböck, 1966). *Hofstenia miamia*, however, shows the epithelium to be at least in part insunk (pers. obs. on Steinböck's type material). As in our species, in most other acoel taxa the pharyngeal cilia are more closely spaced than the epidermal cilia. Representatives of the genus *Hofstenia*, however, are an exception to this in having loosely spaced pharyngeal cilia. Generally, in acoel pharynges the cilia are the same length as the epidermal cilia or longer, as in *Myopea* sp. All these characters stand in contrast to pharyngeal characteristics of Catenulida, Haplopharyngida, and Macrostomida, where the nuclei of epithelial cells are not insunk but lie distal to the pharyngeal musculature and the pharyngeal cilia generally are shorter than the epidermal cilia (Doe, 1981).

There are no detailed ultrastructural data available on the ciliated pharyngeal epithelium of any non-solenofilomorphid acoel taxa, but considering the general uniformity of the structure of locomotory cilia, there seems to be little information for phylogenetic considerations within the Acoela with respect to this character. The ciliary rootlets of the epidermis and pharyngeal cilia in the investigated species show the characteristic features of the acoel ciliary rootlet system with a strong main rootlet and lateral connecting rootlets (Hendelberg and Hedlund, 1974; Tyler, 1984). For *Solenofilomorpha funilis*, Doe (1981) describes a characteristic change in the direction of main ciliary rootlets at the posterior part of the mouth in relation to the ventral epidermis, whereby the rootlets of epidermal cells point rostrally, those of epidermal cells at the mouth point obliquely in a dorsal direction, and those of the posterior pharynx wall point toward the mouth. This orientation allows for an effective stroke of all cilia in the posterior direction, and thus is crucial for ingestion by means of ciliary movement. Our results confirm this relative change in rootlet orientation, but there were no epidermal cells with dorsally directed rootlets at the mouth.

CONCLUSIONS

The acoel pharynx, despite its classification as a pharynx simplex, is a complex organ providing numerous histological and ultrastructural characters informative for comparative investigations. Among these characters, the connection of the pharyngeal musculature to the musculature of the body wall, the presence or absence and (ultra)structure of a posterior nonciliated region, and the ultrastructure of sensory cells are most likely applicable to phylogenetic analyses of acoels and their relationship to other turbellarian taxa. Our data corroborate Doe's (1981) suggestion that the muscular pharynges of Acoela are not homologous to the pharynx simplex in Catenulida and the pharynx simplex coronatus in Macrostomida and Haplopharyngida. At the histological level, comparisons with data available on other acoel taxa with a pharynx even point to the fact that their pharynges may be independently developed, especially considering that there is no pharynx in most Nemertodermatida nor in representatives of the putatively primitive acoel taxon *Paratomella* (see also Doe, 1981). However, this dataset is still fragmentary, detailed descriptions of the mouth and pharynx musculature are often lacking, and there are hardly any ultrastructural data. Thus, additional studies on acoel pharynges applying nontraditional techniques like TEM and fluorescence muscle staining are highly desirable.

ACKNOWLEDGMENTS

We thank Kelly Edwards (Electron Microscopy Laboratory of the Department of Biological Sciences, University of Maine) for technical help, and Matt Hooge for help and discussions.

LITERATURE CITED

- Ax P. 1961. Verwandtschaftsbeziehungen und Phylogenie der Turbellarien. *Ergebn Biol* 24:1–68.
- Ax P. 1996. Multicellular animals. A new approach to the phylogenetic order in nature, vol. I. New York: Springer. p 1–225.
- Ax P, Dörjes J. 1966. *Oligochoerus limnophilus* nov. spec. ein kaspisches Faunenelement als erster Süßwasservertreter der Turbellaria Acoela in Flüssen Mitteleuropas. *Int Rev Ges Hydrobiol* 51:15–44.
- Bock S. 1923. Eine neue marine Turbellariengattung aus Japan. *Uppsala Univ Årsskrift, Mat Och Nat* 1:1–52.
- Crezée M. 1975. Monograph of the Solenofilomorphidae (Turbellaria: Acoela). *Int Rev Ges Hydrobiol* 60:769–845.
- Doe DA. 1981. Comparative ultrastructure of the pharynx simplex in Turbellaria. *Zoomorphology* 97:133–193.
- Dörjes J. 1968. Die Acoela (Turbellaria) der Deutschen Nordseeküste und ein neues System der Ordnung. *Zeitschr Zool Syst Evolution* 6:56–452.
- Dörjes J. 1971. Monographie der Proporidae und Solenofilomorphidae (Turbellaria Acoela). *Senckenb Biol* 52:113–137.
- Hendelberg J, Hedlund KO. 1974. On the morphology of the epidermal ciliary rootlet system of the acoelous turbellarian *Childia groenlandica*. *Zoon* 2:13–24.
- Hooge MD. 2001. Evolution of body wall musculature in the Platyhelminthes (Acoelomorpha, Catenulida, Rhabditophora). *J Morphol* 249:171–194.
- Hyman LH. 1951. Platyhelminthes and Rhynchocoela. The acoelomate Bilateria. In: *The invertebrates*, vol 2. New York: McGraw-Hill. p 1–572.
- Michel C. 1988. Intestine and digestive glands. In: Ax P, editor. *Microfauna marina*, vol. 4. The ultrastructure of Polychaeta. Stuttgart: Gustav Fischer. p 157–175.
- Papi F. 1957. Sopra un nuovo Turbellario arcoforo di particolare significato filetico e sulla posizione della fam. Hofsteniidae nel sistema dei Turbellarii. *Pubbl Staz Zool Napoli* 30:132–148.
- Riedl R. 1954. Neue Turbellarien aus dem mediterranen Felsitoral. *Ergebnisse der "Unterwasser-Expedition Austria 1948–1949."* *Zool Jahrb Syst (Wien)* 82:157–244.
- Rieger RM. 1984. Evolution of the cuticle in the lower Metazoa. In: Bereiter-Hahn J, Matoltsy AG, Richards KS, editors. *Biology of the integument*, vol. 1. Invertebrates. Heidelberg: Springer. p 389–399.
- Rieger R, Sterrer W. 1968. *Megamorion brevicauda*, gen. nov., spec. nov., ein Vertreter der Turbellariengattung Macrostomida aus dem Tiefenschlamm eines norwegischen Fjords. *Sarsia* 31:75–100.
- Rieger RM, Tyler S, Smith JPS, Rieger GE. 1991. Platyhelminthes: Turbellaria. In: Harrison FW, Bogitsh BJ, editors. *Microscopic anatomy of invertebrates*, vol. 3. New York: Wiley-Liss. p 7–140.
- Smith JP III, Tyler S. 1985. Fine structure and evolutionary implications of the frontal organ in Turbellaria Acoela. I. *Dio-pisthoporus gymnopharyngeus* n. sp. *Zool Scr* 14:91–102.
- Steinböck O. 1966. Die Hofsteniiden (Turbellaria Acoela). Grundsätzliches zur Evolution der Turbellarien. *Z Zool Syst Evolution* 4:58–195.
- Sterrer W. 1971. Gnathostomulida: problems and procedures. In: Hulings NC, editor. *Proceedings of the First International Conference on Meiofauna*. Contributions to Zoology 76. Washington, DC: Smithsonian Institution Press. p 9–15.
- Tyler S. 1984. Turbellarian platyhelminths. In: Bereiter-Hahn J, Matoltsy AG, Richards KS, editors. *Biology of the integument*, vol. I. Invertebrates. New York: Springer. p 112–131.
- Westblad E. 1940. Studien über skandinavische Turbellaria Acoela. I. *Ark Zool* 32:1–28.
- Westermann B, Schipp R. 1998. Cytological and enzyme-histochemical investigations on the digestive organs of *Nautilus pompilius* (Cephalopoda, Tetrabranchiata). *Cell Tissue Res* 293:327–336.